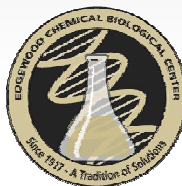




Decreased Mitotic Potency of Sarin Vapor Following Multiple Low-level Inhalation Exposures

Paul Adam Dabisch, Ph.D.
NRC Postdoctoral Associate
US Army Edgewood Chemical Biological Center



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Purpose

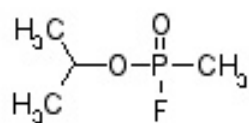
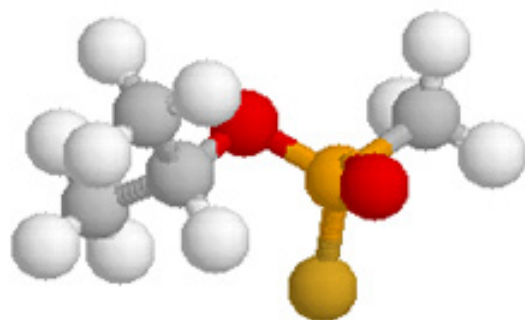
To determine the effect of multiple low-level exposures to sarin vapor on the eye. Endpoints investigated included:

- pupil diameter
- ocular cholinesterase activity
- light reflex
- effect of muscarinic receptor blockade
- effect of sympathetic blockade



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Background



Sarin (GB)

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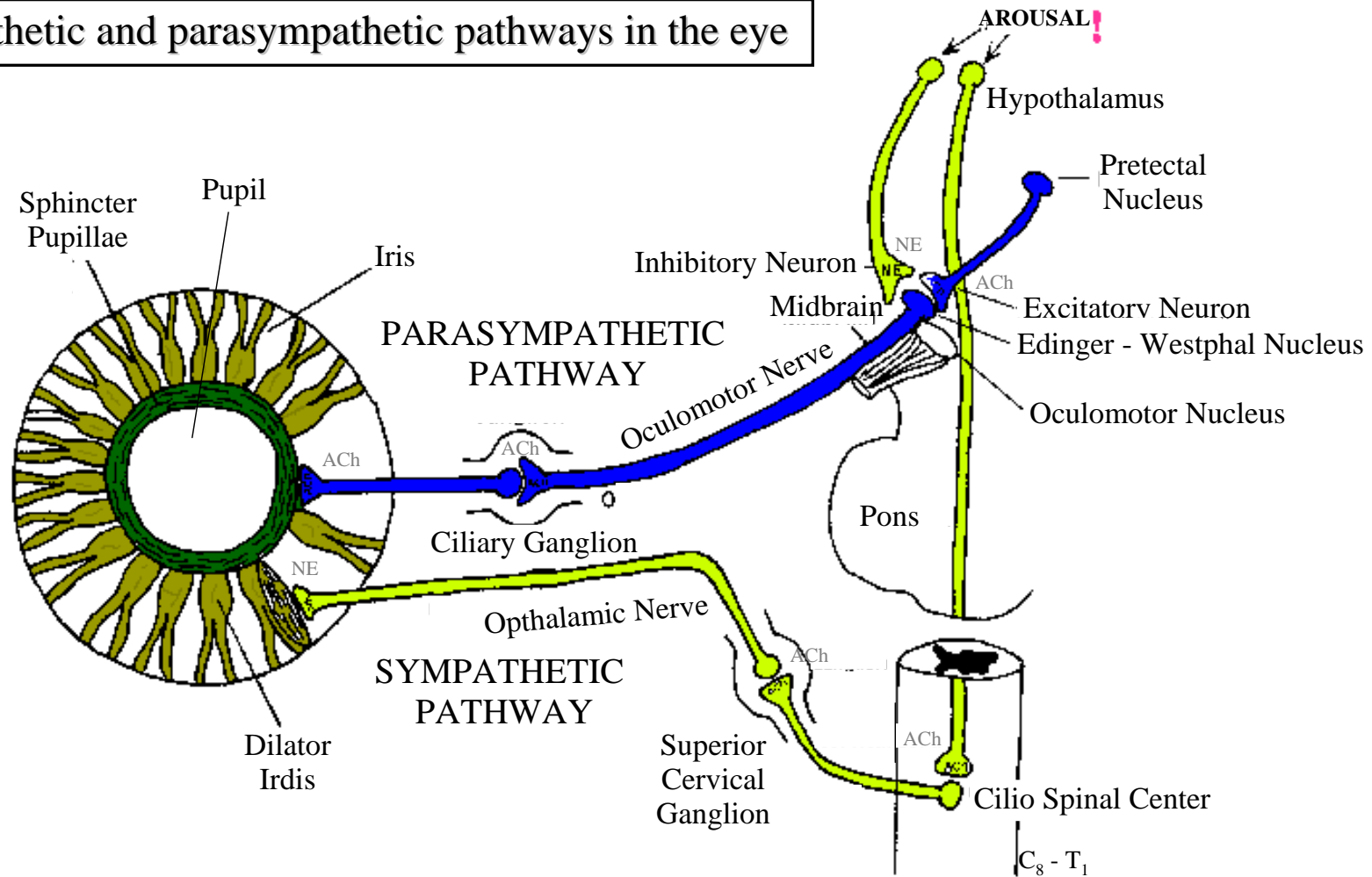
- Also known as German Nerve Agent B, or GB.
- Organophosphorus nerve agent
- More volatile than GA, GD, or VX → greater inhalation hazard



ECRC

Innervation of the eye

Sympathetic and parasympathetic pathways in the eye





ECRC

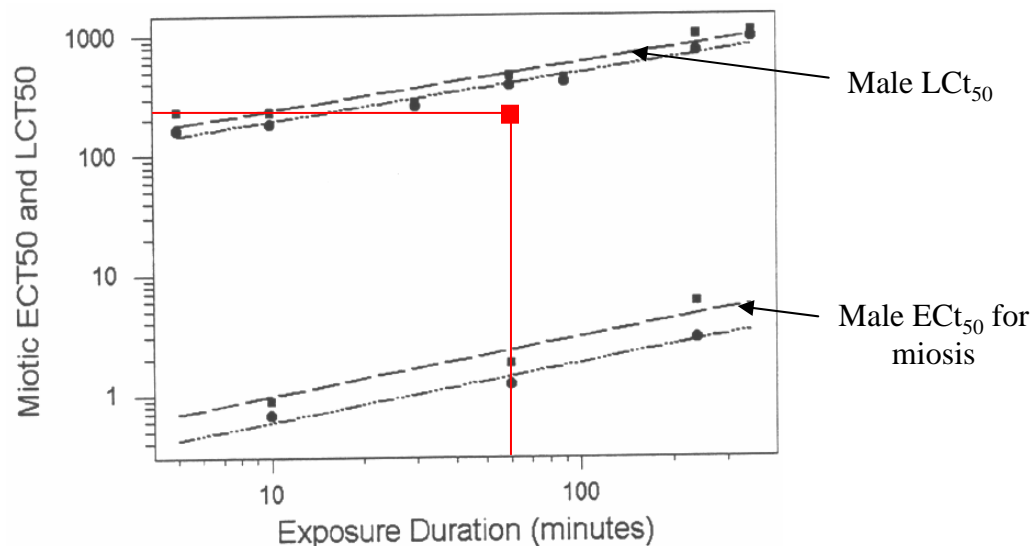
Experimental Design

- Adult male Sprague-Dawley rats were exposed to GB vapor in a 750-L dynamic airflow chamber.
- GB vapor was generated using a spray atomization system.
- Chamber concentrations were determined using thermal desorption tubes (Tenax-TA) and GC-FID analysis.



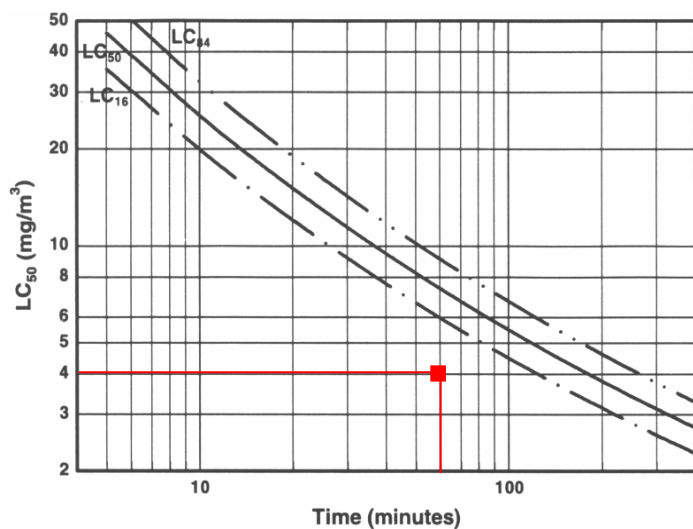


Experimental Design



- Rats were exposed to GB vapor for 1 hour on each of 3 consecutive days. Exposures occurred 24 hours apart

- Exposure concentration was 4.0 mg/m³



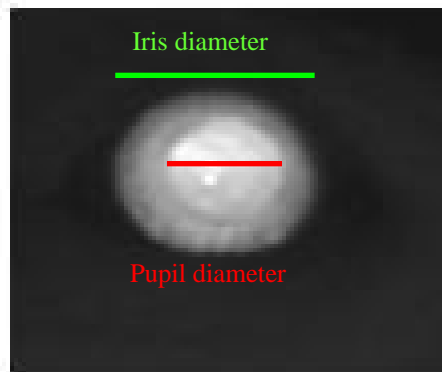
- The concentration of GB vapor chosen was well above the EC₅₀ for miosis (0.030 mg/m³ for a 60 minute exposure), but below the LC₅₀ (7.7 mg/m³ for a 60 minute exposure).
Mioduszeewski et al. 2001, 2002



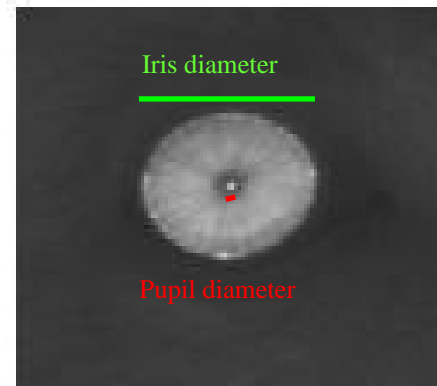
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GB-induced Miosis

One of the first effects seen in a vapor exposure to GB is miosis.



Pre-exposure



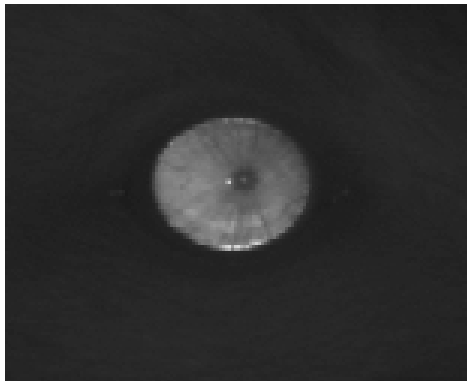
Post-exposure

- GB vapor exposure (4.0 mg/m^3) for 1 hour
- Pupil images acquired with an IR capable camera
15 minutes post-exposure

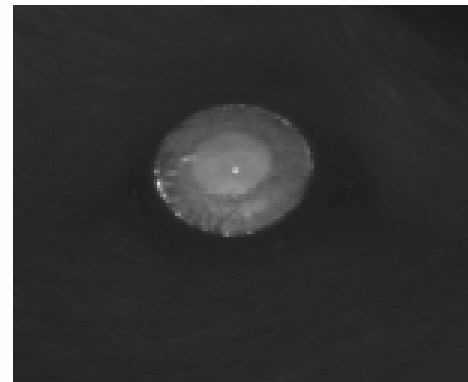


Atropine Pre-treatment

- Previously, it has been suggested that nerve agent induced miosis is a local effect (Soli et al. 1980).
- In the present study, atropine (6 mg/kg i.m.) blocked GB-induced miosis.



No pre-treatment

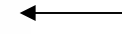
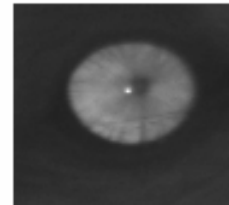
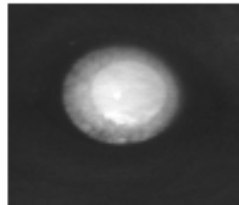
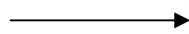


Atropine pre-treatment



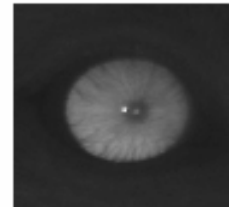
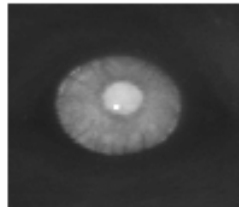
Development of Mitotic Tolerance

Baseline



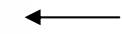
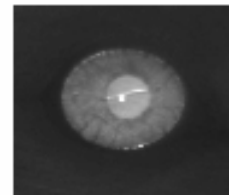
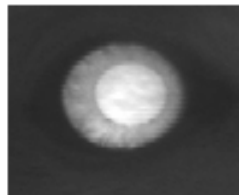
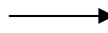
15 minutes post
exposure #1

1 hour pre-
exposure #2



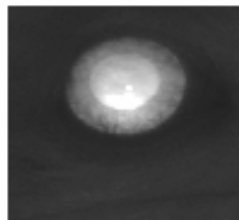
15 minutes post
exposure #2

1 hour pre-
exposure #3



15 minutes post
exposure #3

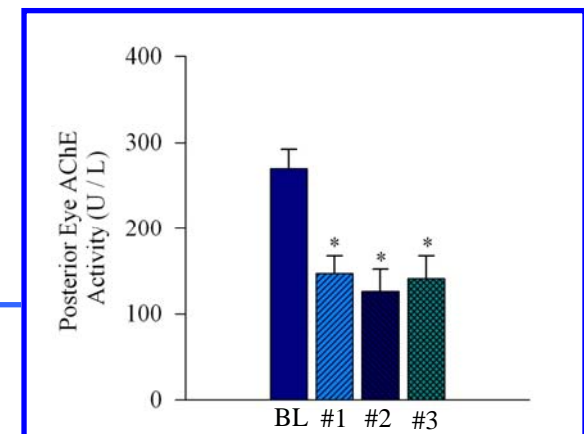
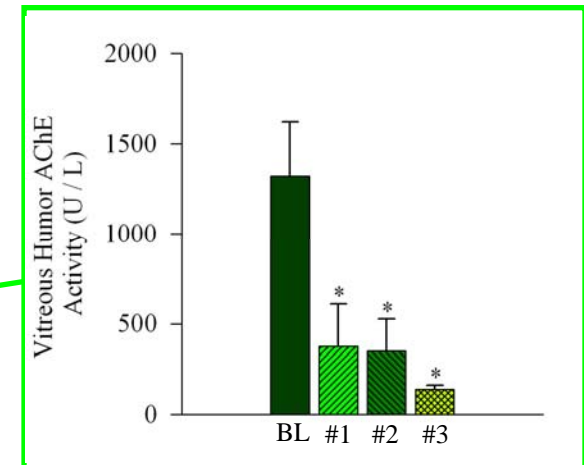
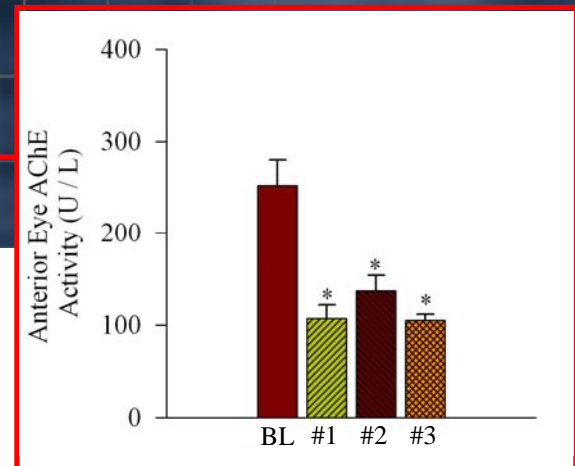
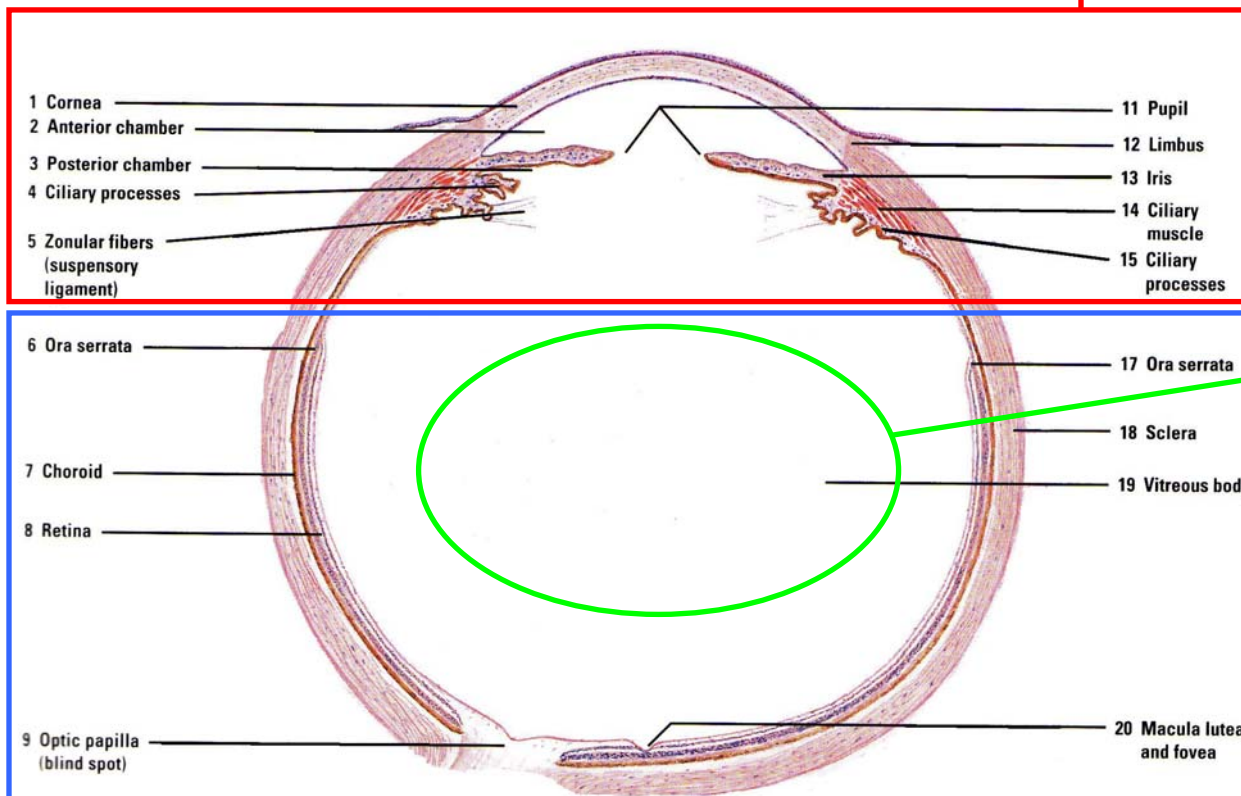
24 hours post-
exposure #3





Ocular AChE Activity

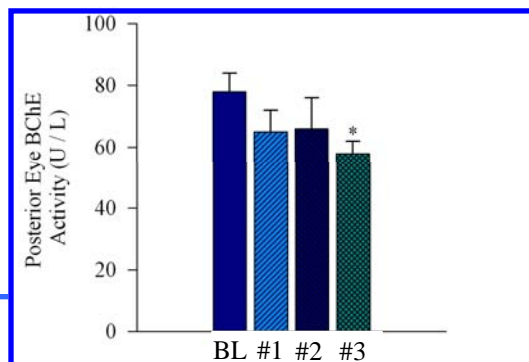
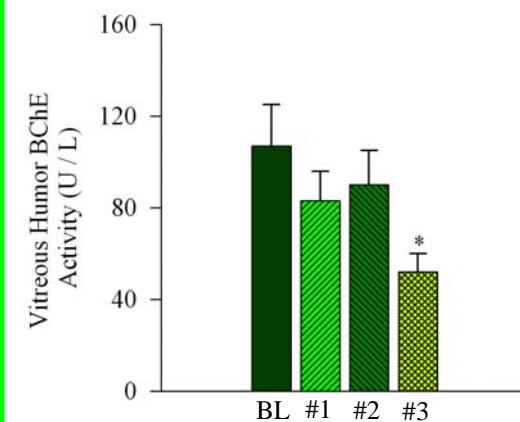
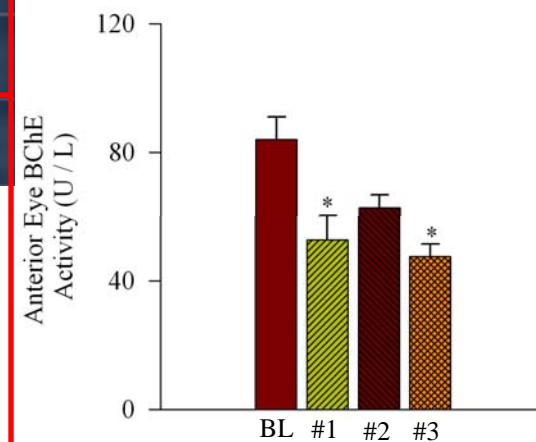
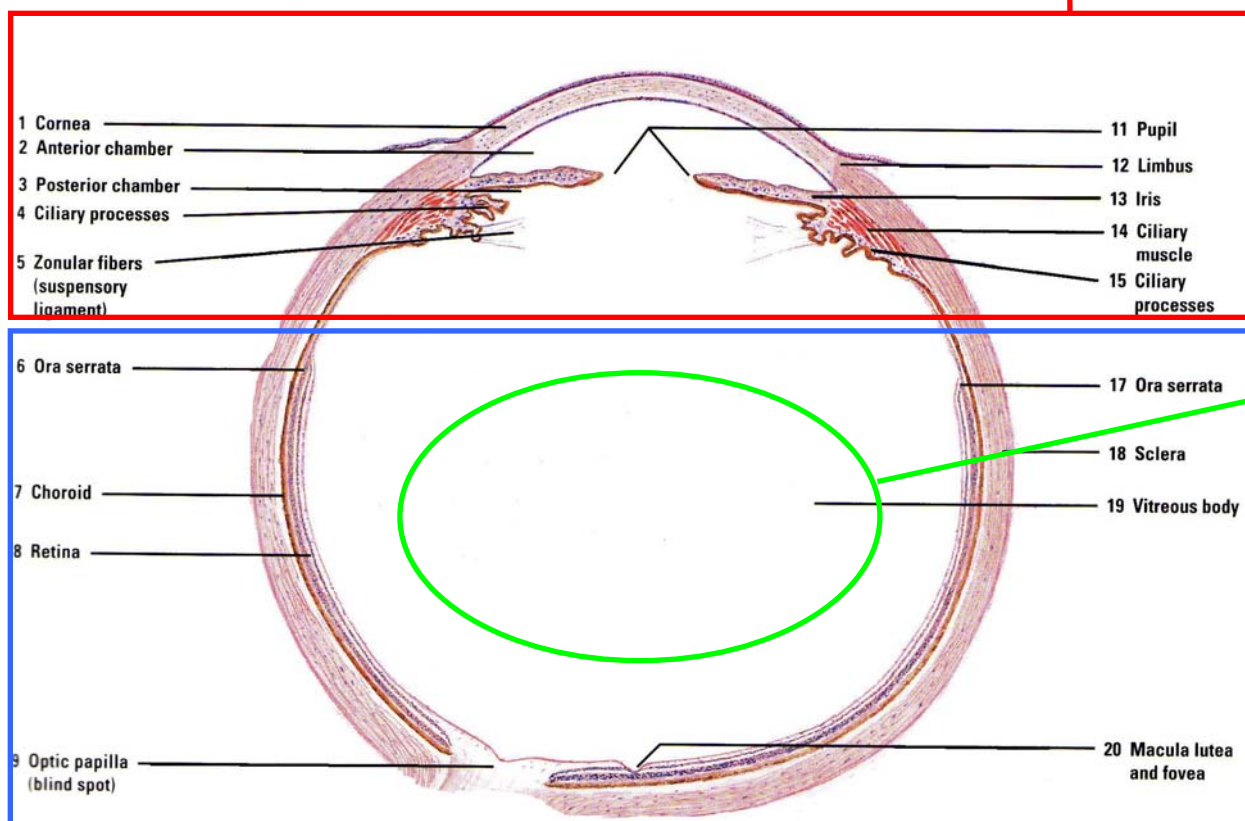
EYE (SAGITTAL SECTION)





Ocular BChE Activity

EYE (SAGITTAL SECTION)



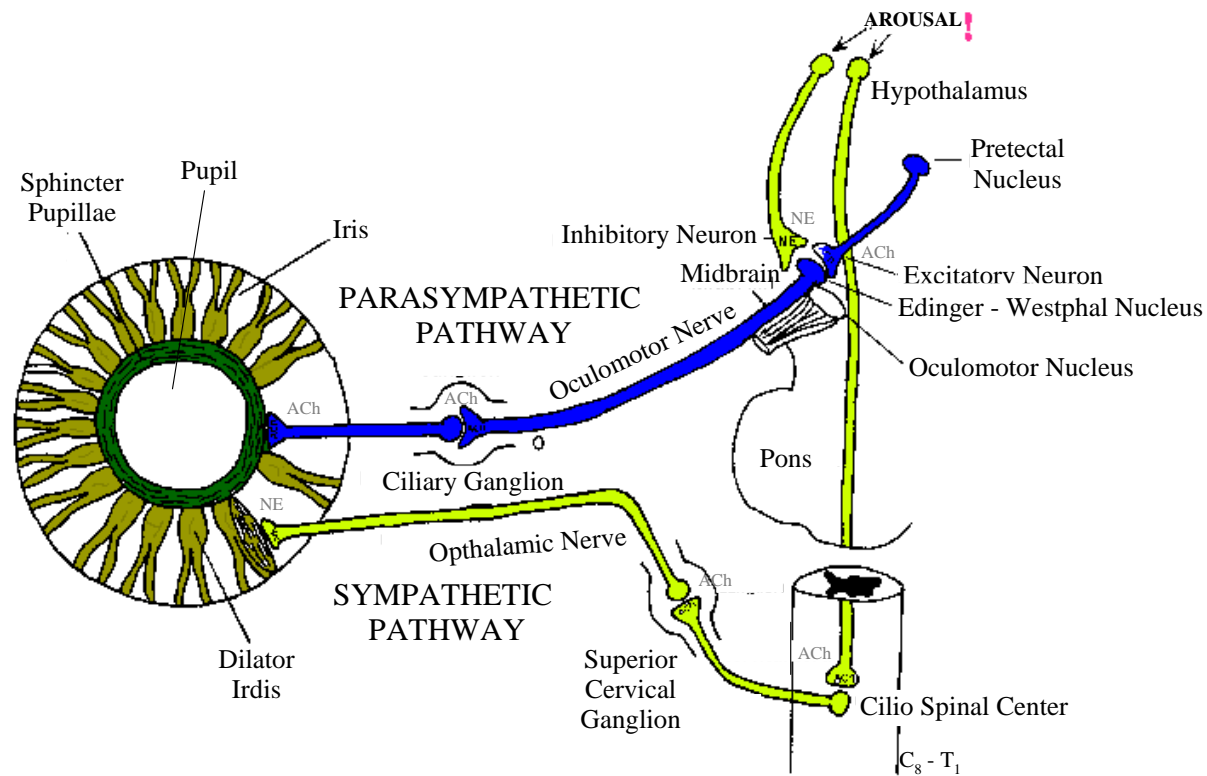


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Hypotheses

2 hypotheses:

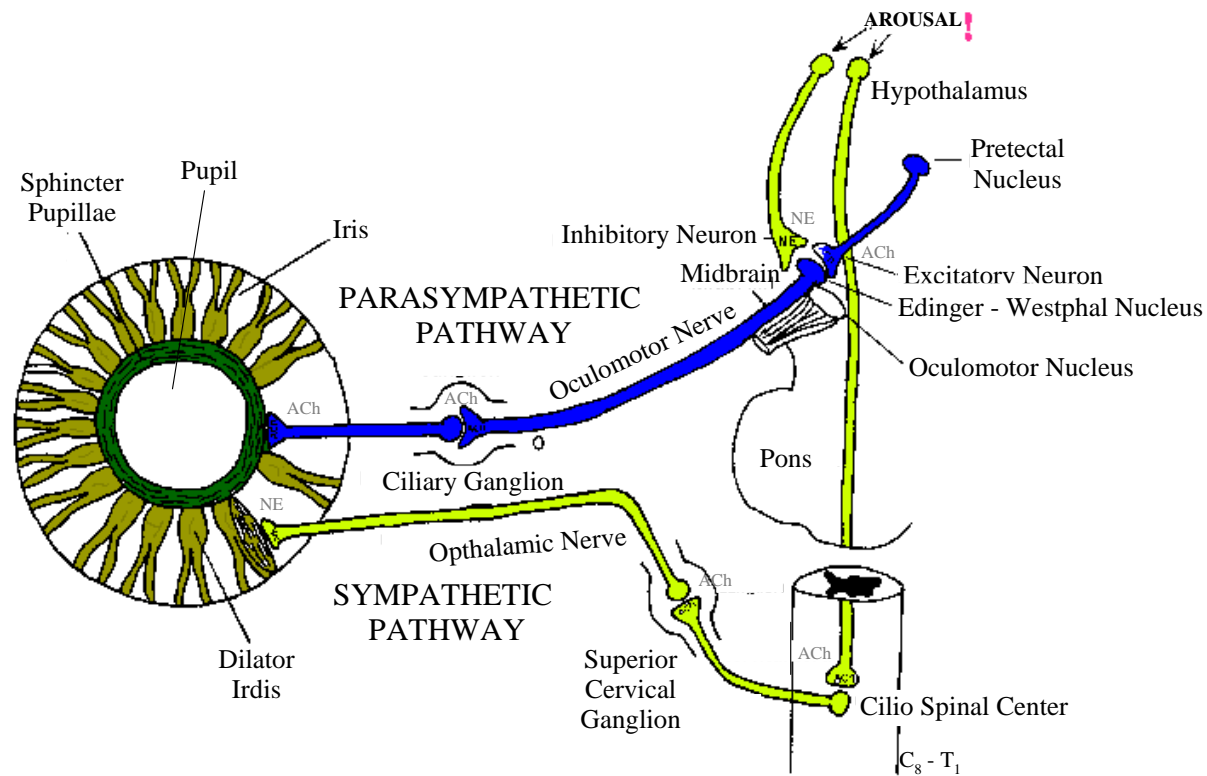
- 1 – The observed tolerance is due to increased sympathetic tone in the iris, resulting in a lesser degree of miosis.





Experimental Design

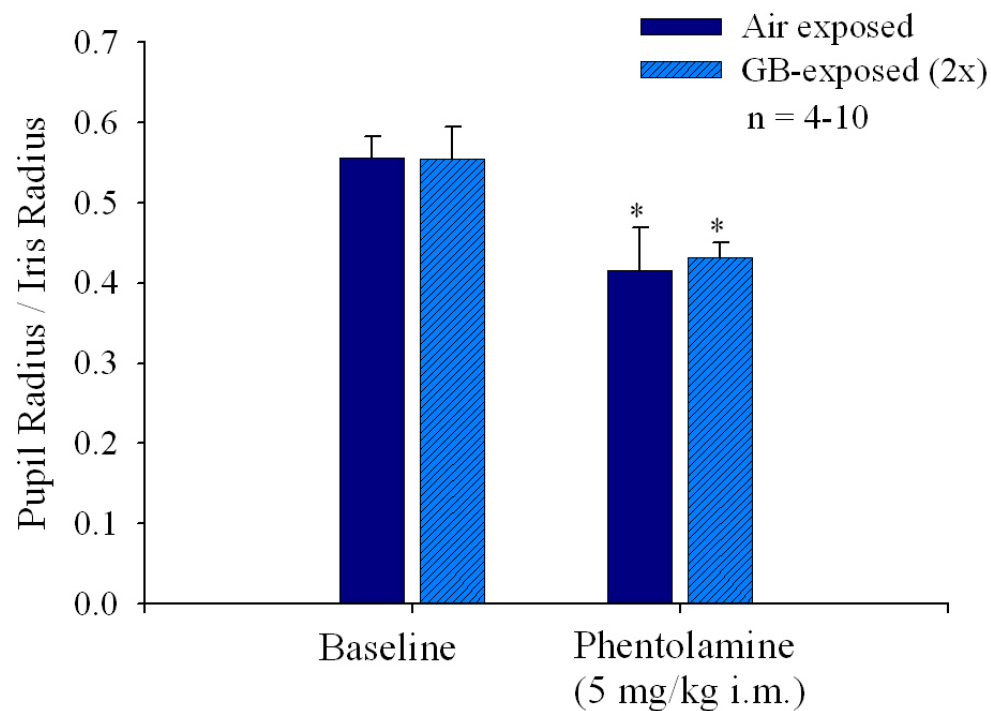
- Rats were administered **phentolamine**, a non-selective α -adrenergic receptor antagonist.





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Results

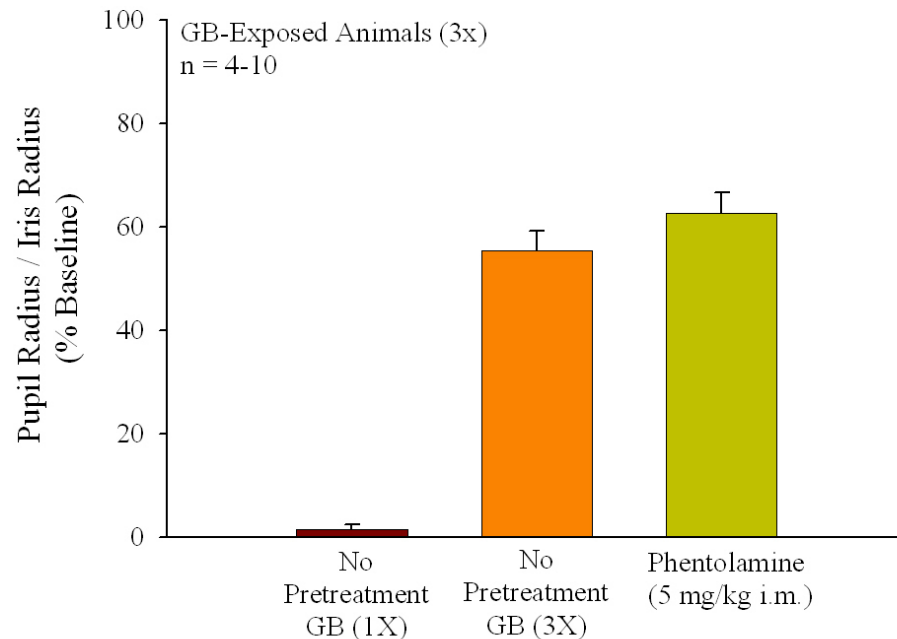


- Phentolamine administration produced similar decreases in the ratio of pupil radius to iris radius in both air- and GB-exposed animals.
- This suggests that baseline sympathetic tone was similar in air- and GB-exposed animals.
- Similar results were obtained with propranolol and the combination of phentolamine and propranolol (Data not shown).



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Results



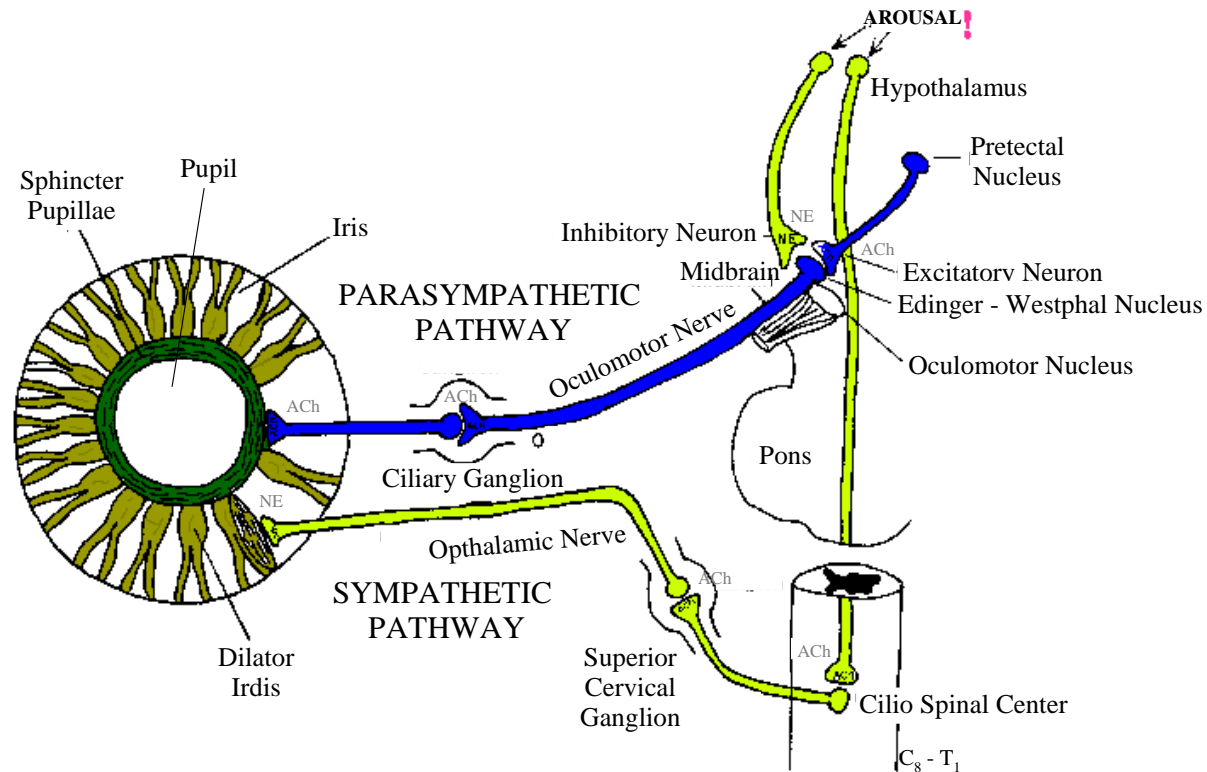
- Phentolamine pretreatment did not affect the development of mitotic tolerance
- This suggests that the tolerance is not mediated by an enhancement alpha-receptor mediated sympathetic tone.
- Similar results were obtained with the combination of propranolol and phentolamine (Data not shown).



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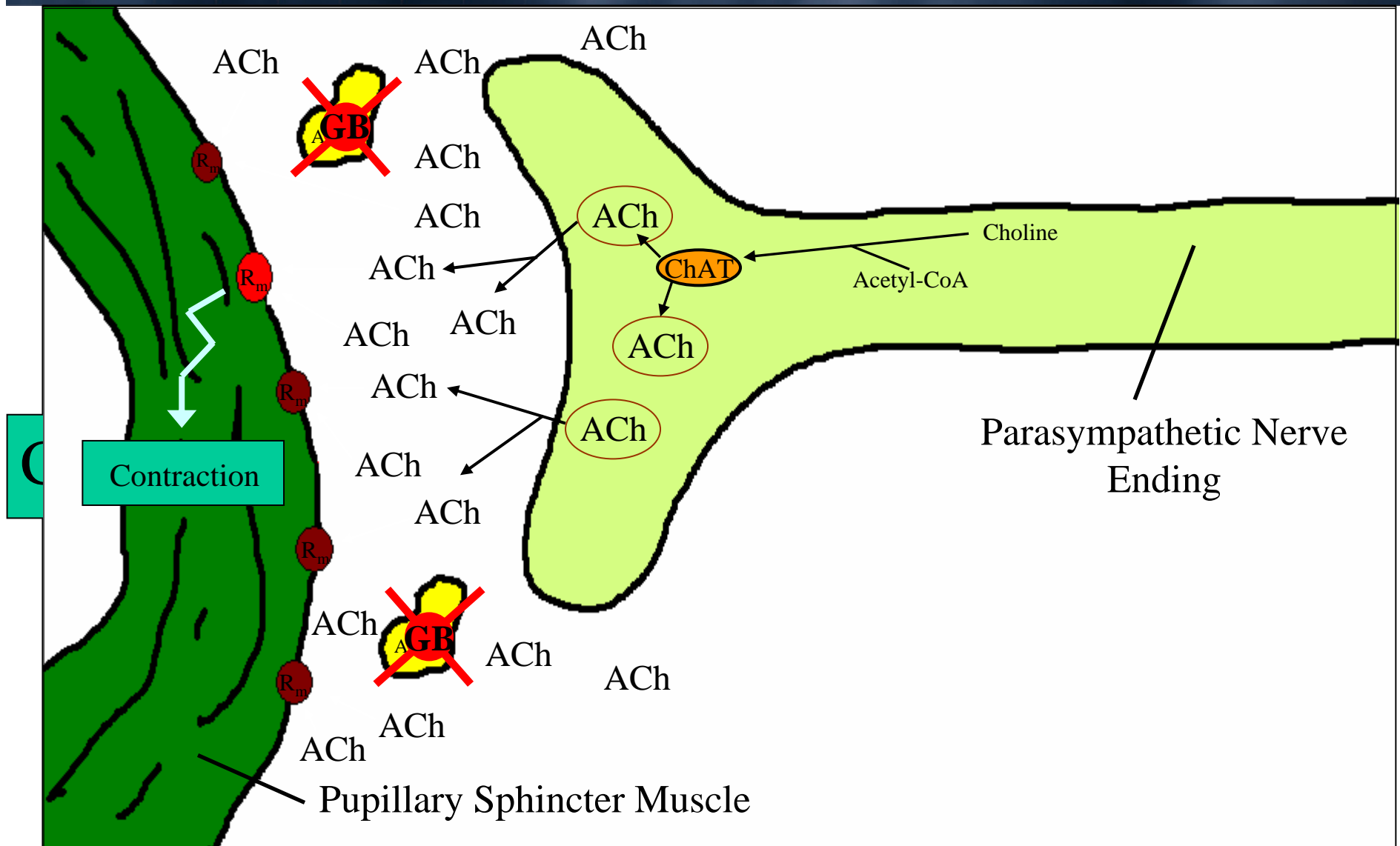
Hypotheses

2 – The observed tolerance is due to desensitization of muscarinic receptors secondary to excessive cholinergic stimulation.





Prolonged GB exposure



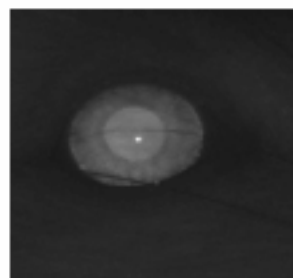


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Results

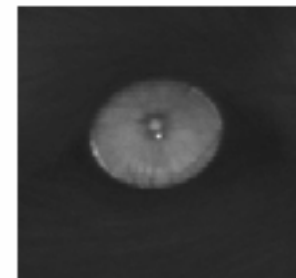
Post-Exposure #3

- Atropine pre-treated animals had pinpoint pupils following exposure, whereas untreated animals only had a 40% reduction in the pupil:iris ratio.



GB-exposed

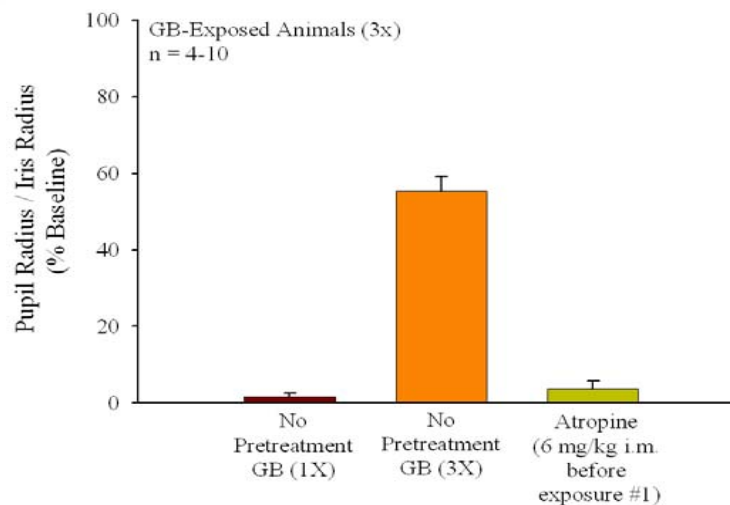
No pre-treatment



GB-exposed

Atropine pre-treatment

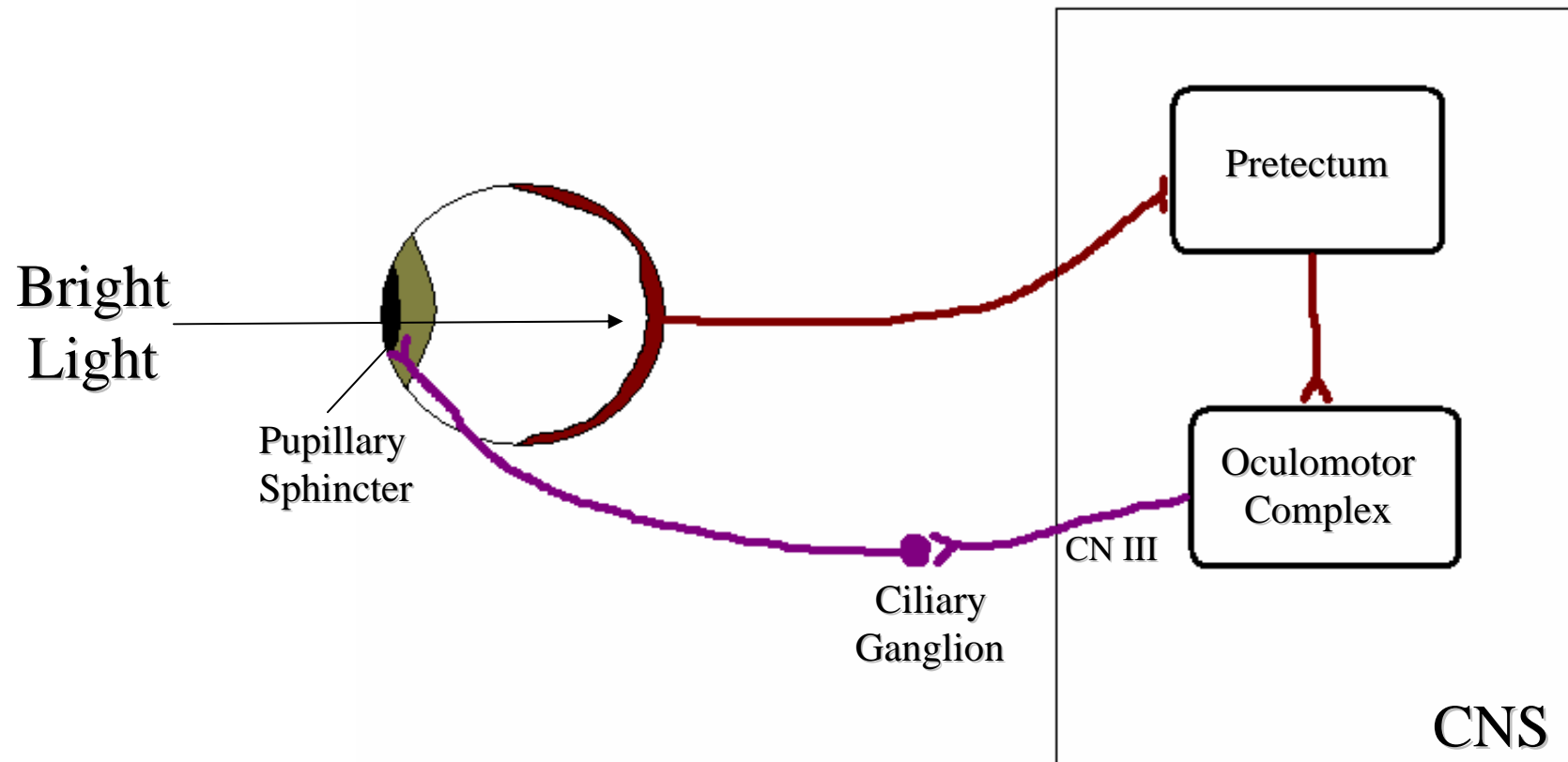
- These data demonstrate that atropine is able to prevent the miotic tolerance observed in rats exposed to GB vapor multiple times.





ECPC

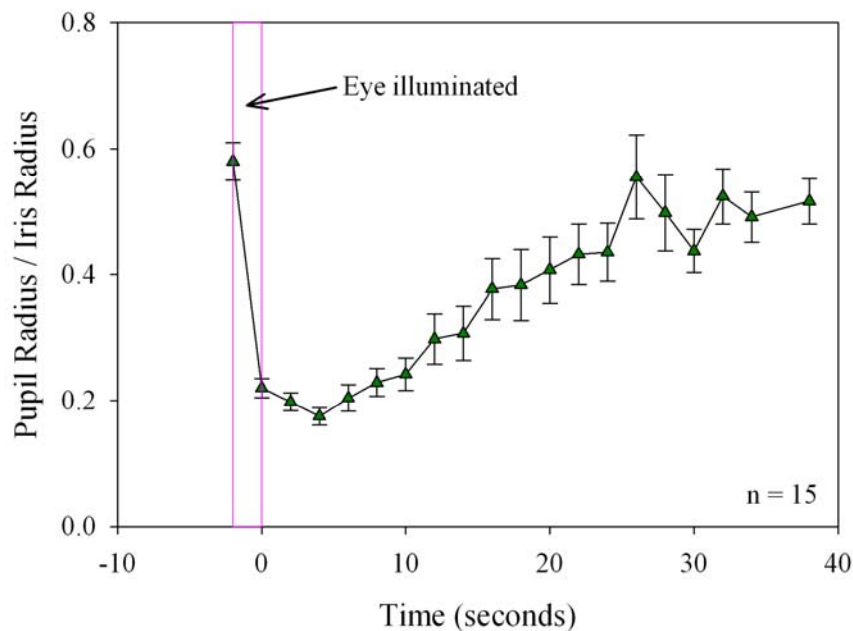
Pupillary Light Reflex



- Bright light sensed by the retina starts a reflex arc, which ultimately constricts the pupil.

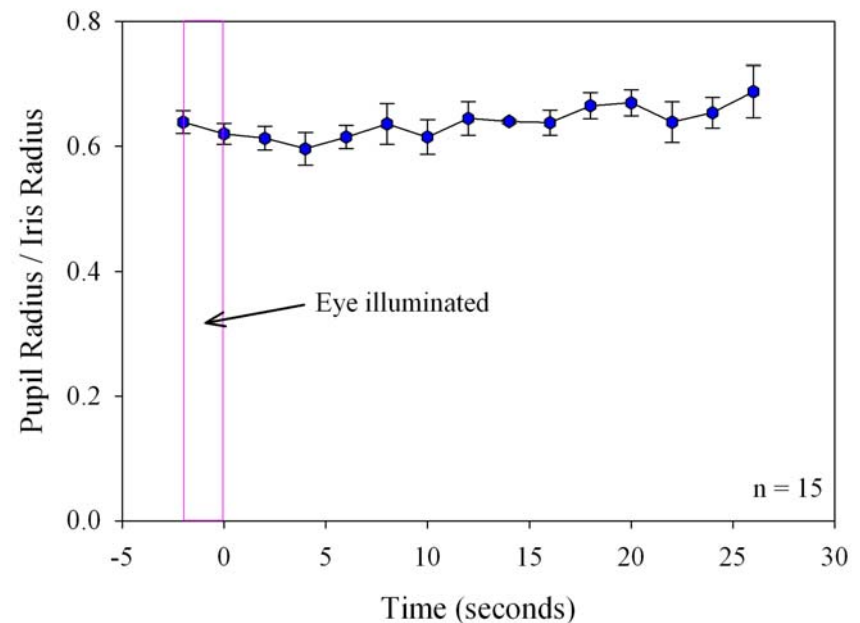
Loss of the Pupillary Light Reflex

Air-exposed animals



GB-exposed animals

(2 x 240 mg • min / m³)

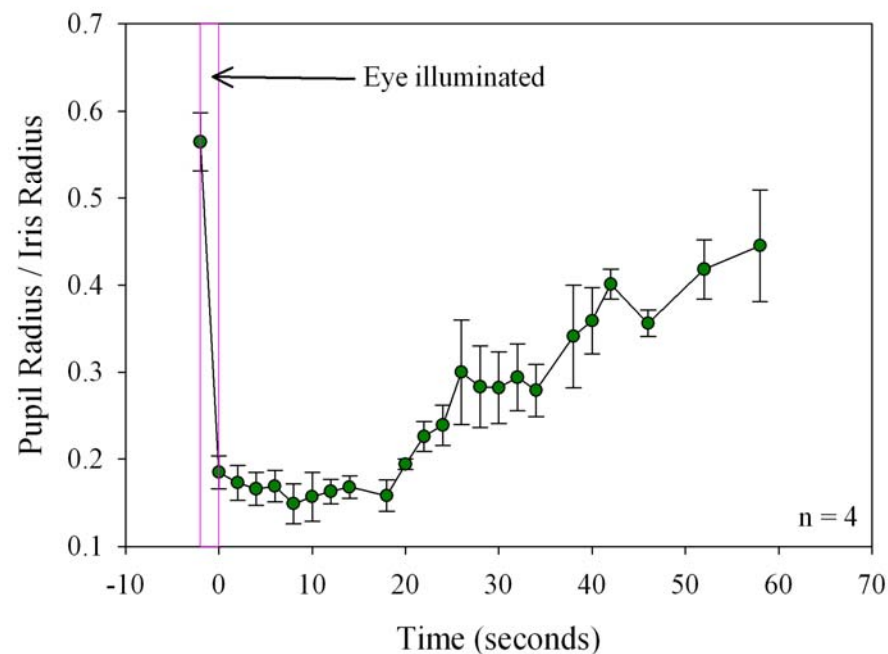


- Following exposure #2 (20 hours post), the light reflex is absent in GB-exposed animals.



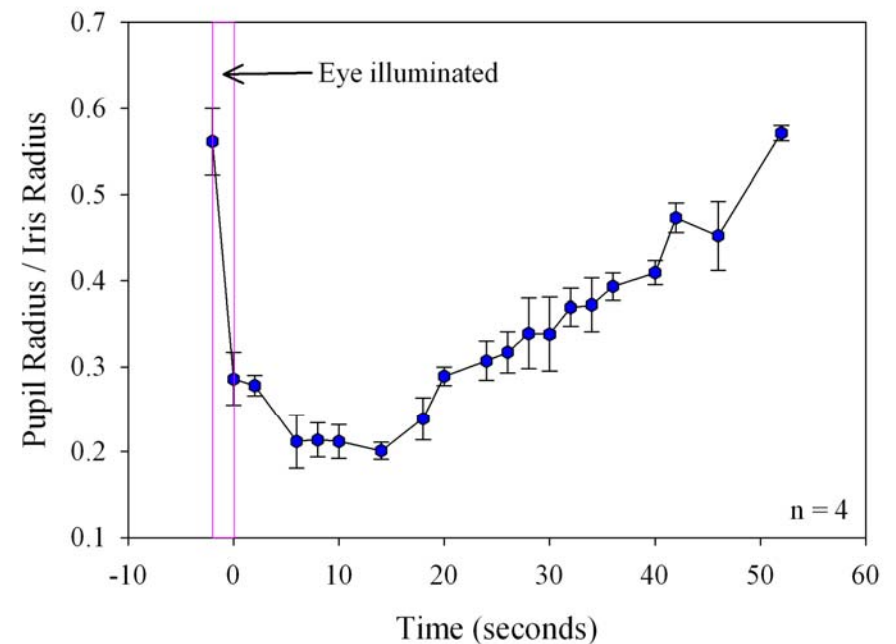
Loss of the Pupillary Light Reflex

Air-exposed animals



GB-exposed animals

(3 x 240 mg • min / m³)



- 96 hours post exposure #3, the light reflexes in GB-exposed and air-exposed animals are similar.



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Summary

- Tolerance to the miotic effect of GB vapor occurs following multiple exposures, and lasts for ~4 days.
- The tolerance is not likely due to a decreased inhibitory effect of GB, or enhanced sympathetic tone to the eye.
- This tolerance is likely due to the desensitization of muscarinic receptors located on the pupillary sphincter muscle.



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